

Session 4

Plasmid Mini-Preparation & Restriction Digestion

Learning Objective:

The goal of this exercise is to become familiar with the procedure for isolating plasmid DNA from bacteria and running a restriction endonuclease digestion.

Introduction

Our ability to engineer biology depends on our ability to move DNA into and out of cells; today we will focus on out. Isolating small DNA (plasmids) from cells is a frequent procedure in molecular biology. Vector sources are maintained in strains for ease of mass production through culturing. Vectors maintained in strains and isolated for use need to be prepared to accept insert DNA. This is done by restriction digestion. Restriction digestion will cut a circular plasmid to make it linear; leaving ends which are compatible with other pieces of DNA cut with the same endonucleases. We will use this principle to prepare both our vector and PCR amplified product.

Background

Isolating DNA from a Cell

Removing DNA from a cell is a relatively simple process, especially when it comes to small plasmid DNA. The first step in this process is to break open the cells in a process called lysis. In this step, the cell membrane is disrupted with the use of a detergent and base and sometimes the use of physical means like sonication or pressing. Insoluble cell debris is then removed from the solution containing the soluble DNA. The next step involves purifying the DNA to remove unwanted proteins and salt. This is done by using isopropanol and ethanol to precipitate the DNA to an insoluble form and by selectively binding the DNA to silica beads. With the DNA firmly tied up, it can be washed to remove impurities. The final step is to elute the DNA from silica beads and re-dissolve it in water or the desired buffer solution. While the exact steps of this procedure vary depending on the location and size of the DNA and the type of cells being used, the basic steps are the same: break open the cells, purify the DNA, and re-suspend the DNA.

Restriction Endonucleases

An *enzyme* is a protein which catalyzes a chemical reaction. A restriction endonuclease is an enzyme which cleaves DNA in a specific location which is referred to as a *recognition sequence*. Recognition sequences can vary in length but are typically around six basepairs. Recognition sequences are typically inverted repeat palindromes, this means that the sequence reads the same regardless of the DNA strand on which it is located. For example the sequence:

5'-TCTAGA-3'

will have a complementary sequence of:

3'-AGATCT-5'

When the complementary sequence is read in the proper 5' to 3' orientation (right to left

in this case) it is identical to the original sequence (read left to right). Different enzymes can share the same recognition sequence, these are called *isoschizomers* if they cut the DNA in the same location, and *neoschizomers* if they cut the DNA in different locations.

Depending on how and where the enzyme cuts the DNA, the result will be different. For instance the sequence 5'-GGGCCC-3' can be cut in the middle on both strands by one enzyme (SmaI) to produce two new fragments, 5'-GGG-3' and 5'-CCC-3'. Alternatively, the same sequence can be cut by a different enzyme (XmaI) to produce different ends, 5'-G-3' and 5'-GGCCC-3'. Because the cut location is in the same relative location of the sequence on both strands, the results of the two cuts are very different:

Original: 5'-GGGCCC-3'
 3'-CCCGGG-5'

<p>SmaI: 5'-GGG-3' + 5'-CCC-3'</p> <p style="padding-left: 40px;">5'-CCC-5' 3'-GGG-3'</p>	<p>XmaI: 5'-G-3' + 5'-GGCCC-3'</p> <p style="padding-left: 40px;">3'-CCCGG-5' 5'-G-3'</p>
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The cut produced by SmaI is referred to as a **blunt-end**, while the cut from XmaI produces an **overhang** sequence on one of the DNA strands which is referred to as a **sticky-end** or **cohesive-end**. The overhangs are called “sticky” because, despite no longer being covalently bonded, the two strands “prefer” to remain paired because of favorable thermodynamic energetics, meaning they spend a great amount of time paired than unpaired.

It is possible for overhangs produced from different enzymes cutting different recognition sequences to produce ends with compatible sticky ends. As an example:

<p>XbaI: 5'-TCTAGA-3'</p> <p style="padding-left: 40px;">3'-AGATCT-5'</p>	Cuts to	<p>5'-T-3'</p> <p style="padding-left: 40px;">5'-AGATC-5'</p>	+	<p>5'-CTAGA-3'</p> <p style="padding-left: 40px;">3'-T-3'</p>
<p>SpeI: 5'-ACTAGT-3'</p> <p style="padding-left: 40px;">3'-TGATCA-5'</p>	Cuts to	<p>5'-A-3'</p> <p style="padding-left: 40px;">5'-TGATC-5'</p>	+	<p>5'-CTAGT-3'</p> <p style="padding-left: 40px;">3'-A-3'</p>

Notice that the overhang regions by both cuts are the same (5'-GATC-3'). Therefore, these overhangs are complementary and can pair just as well as those from just one cut. However, if the two sequences are joined permanently, neither restriction enzyme is capable of cutting the product. This **scar**, shown below, is the result of changing the six-base sequence so that it is no longer a palindrome and therefore not recognized by either enzyme.

<p>XbaI + SpeI:</p>	<p>5'-T-3'</p> <p style="padding-left: 40px;">3'-AGATC-5'</p>	+	<p>5'-CTAGT-3'</p> <p style="padding-left: 40px;">3'-T-3'</p>	=	<p>5'-TCTAGT-3'</p> <p style="padding-left: 40px;">3'-AGATCA-5'</p>
<p>SpeI + XbaI:</p>	<p>5'-A-3'</p> <p style="padding-left: 40px;">3'-TGATC-5'</p>	+	<p>5'-CTAGA-3'</p> <p style="padding-left: 40px;">3'-T-3'</p>	=	<p>5'-ACTAGA-3'</p> <p style="padding-left: 40px;">3'-TGATCT-5'</p>

Session 4: Pre-Laboratory Exercises

Name: _____

Date: _____

- 1) How is DNA isolated from cells?

- 2) What is an enzyme?

- 3) What does a restriction endonuclease do?

- 4) What are “sticky-ends” and why might they be better to use for cloning than “blunt-ends”?